Cancer can be defined as an unregulated growth of cells arising from a single cell. The scientific or medical term for cancer is malignant neoplasm, which is defined as a relatively autonomous growth of tissue not subject to the rules and regulations of normal growing cells. Tumor is a general term indicating any abnormal mass or growth of tissue. Therefore, a neoplasm is a tumor. Major features of benign tumors are encapsulation, slow growth, and non-invasion of surrounding tissue; that is, lack of metastasizing ability. Malignant tumors grow rapidly, are not encapsulated and invade surrounding tissue and metastasize. Benign growths generally have a normal complement of chromosomes, exhibit good differentiation, and have rare cell division. The opposite is characteristic of malignant neoplasms. Between the two extremes of fully normal and highly malignant tissue architectures lies a broad spectrum of tissues of intermediate appearance. The different gradations of abnormality may well reflect cell populations that are evolving progressively away from normal and toward greater degrees of aggressive and invasive behavior. Pathology grading systems classify the microscopic cell appearance abnormality, deviations in their rate of growth, degree of invasiveness and dissemination with the goal of predicting developments at tissue level in the sampled tumors. The four broad histological grades are hyperplasia, dysplasia, Carcinoma in situ (CIS) and Invasive carcinoma.

**Hyperplasia** is a common preneoplastic response to stimulus. Microscopically cells resemble normal cells but are increased in numbers.

**Dysplasia** is a term used in pathology to refer to an abnormality of development. This generally consists of an expansion of immature cells, with a corresponding decrease
in the number and location of mature cells. Dysplasia is often indicative of an early neoplastic process.

**Carcinoma in situ** (CIS), meaning "cancer in place," represents the transformation of a neoplastic lesion to one in which cells undergo essentially no maturation, and thus may be considered cancer-like. In this state, epithelial cells have lost their tissue identity and have reverted back to a primitive cell form that grows rapidly and without regulation.

**Invasive carcinoma** is the final step in this sequence. It is a cancer which has invaded beyond the basement membrane and has potential to metastasize (spread to other parts of the body) [Pazdur et al., 2008].

Epithelial tissues are of special interest here, **Epithelia** are sheets of cells that line the walls of cavities and channels or, in the case of skin, serve as the outside covering of the body, because they spawn the most common human cancers—the **carcinomas**. These tumors are responsible for more than 80% of the cancer-related deaths worldwide [Ferlay et al., 2001]. Included among the carcinomas are tumors arising from the epithelial cell layers of the gastrointestinal tract—which includes mouth, esophagus, stomach, and small and large intestines—as well as the skin, mammary gland, pancreas, lung, liver, ovary, gallbladder, and urinary bladder. Although cancer is usually regarded as a problem of the developed world, about two-thirds of all cancer occurs in the three-quarters of the world’s population who live in developing countries. According to GLOBOCAN 2008 worldwide, there are about 22 million people living with cancer at any one time. Further, the number of cases of cancer worldwide is predicted to increase by 5 million to 15 million new cases each year by 2020 [Ferlay et al., 2010].
Incidence of Oral and Esophageal Cancer

Oral cancer is the cancer of the mouth. The term “oral” includes the lips and all intra-oral sites corresponding to the ICD 9 codes 140 (lip), 141 (tongue), 143 (gum), 144 (floor of the mouth) and 145 (other non-specific sites), but excludes sites 142 (major salivary glands), 146 (oropharynx), 147 (nasopharynx), 148 (hypopharynx) and 149 (ill defined oral/oropharynx) [Johnson et al., 1993]. Approximately 90% of the carcinomas of the oral cavity are squamous cell carcinomas arising from the lining mucosa of the mouth, most commonly the cheek, tongue and the floor of the mouth [Part et al., 1998].
Figure 1.1 A: GLOBOCAN 2008 Map of Cancer (INDIA) for Men

India: Men

Estimated number of cancer deaths, all ages (total: 321,384)
India: Women
Estimated number of cancer cases, all ages (total: 518,762)

Figure 1.1 B: GLOBOCAN 2008 Map of Cancer (INDIA) for Women
The first description about oral cancer appears in an ancient Indian surgical text written in Sanskrit, *Sushruta Samhita*, around 600BC. Literary references to the habit of chewing betel quid (betel leaf, areca nut and lime) in India are at least 2,000 years old. Tobacco was introduced around the sixteenth century. Some of the first hypotheses on oral cancer were recorded, also in India, in 1902 where betel quid use was suspected to play a role [Boyle et al., 1990]. Oral Squamous Cell Carcinoma (OSCC) is ranked tenth leading site of cancer worldwide with approximately 5,50,765 new cases diagnosed in the year 2008 and 3,50,683 deaths for a mortality of 55%. In the same year India witnessed an estimated of 69,280 new cases of OSCC diagnosed, and this malignancy in total accounts for the deaths of approximately 47,653 patients. In India, it is ranked 2nd with respect to new cases reported (45,445 in 2008) and 3rd with respect to mortality (31,102) in male patients whereas in female patients it is ranked 4th (incidence) and 5th (mortality), respectively [Ferlay et al., 2010] (Fig. 1.1 A & B).

The Esophagus is the upper tract of the digesting system connecting the throat to the stomach and allows the passage of food and fluid to the stomach. It is approximately 10 inches long in adults. The Esophagus is comprised of a mucosa, a submucosa, a muscularis and an adventitia. The mucosa is made up of epithelium, lamina propria, and muscularis mucosae. The epithelium consists of stratified epithelial cells. There are also esophageal glands embedded in the submucosa. There are two major types of cancer of the esophagus, squamous cell carcinoma and adenocarcinoma. The top layer of the lining of the entire length of the esophagus is made up of squamous cells, so squamous cell carcinoma can begin anywhere in the organ. Adenocarcinoma, on the other hand, starts only in the lower part of the esophagus, near the opening of the
stomach, where glandular tissue is present. Prior to the development of adenocarcinoma, the squamous cells near the opening of the stomach must be altered by acid reflux as in Barrett’s esophagus. Esophageal squamous cell carcinoma (ESCC) develops through a progressive sequence from mild to severe dysplasia, carcinoma in situ and, finally, invasive carcinoma [Anani et al., 1991]. The tumors are present as ulcerating or infiltrating lesions in the esophageal epithelium. Most esophageal cancer patients present with advanced metastatic disease at the time of diagnosis [Layke and Lopez, 2006]. This results in a poor prognosis; only 1 in 5 esophageal cancer patients survive more than 3 years after initial diagnosis [Polednak, 2003; Younes et al., 2002]. Studies in high-risk areas point to specific environmental factors as etiological agents of ESCC. Globally 4, 82,239 new cases of esophageal cancer were reported in 2008 and this disease caused the loss of 4, 06,806 lives in the same year. It is ranked among male patients as the 6th leading cancer site (by Incidence) and 5th (by Mortality) worldwide. In India the trend of ESCC is similar to the global pattern of incidence and mortality. A total of 48,099 new cases and 43,351 deaths were recorded in the year 2008. The geographical variation in incidence for esophageal cancer is probably larger than for any other type of cancer, suggesting the influence of environmental factors [Parkin et al., 2005]. In the East and in Africa, the main etiological factors involved have been a diet contaminated with nitrosamines and mycotoxins and deficient in anti-oxidants [Dutton, 1996] and in the Indian subcontinent tobacco chewing and smoking and betel nut chewing are the major factors. In the developed industrialized countries, the main etiological factors involved are alcohol consumption and tobacco smoking [Castellsague et al., 1999]. Drinking hot tea, a habit common in Golestan province of Iran was strongly
associated with a higher risk of oesophageal cancer [Islami et al., 2009]. Studies in high-risk areas have also demonstrated a strong tendency towards familial aggregation or clustering of cases within families, suggesting that genetic susceptibility factors may also play an important role in the etiology of ESCC [Engel et al., 2003].

Etiology of these Cancers

Epidemiological studies have clearly indicated that Betel - quid chewing, cigarette smoking and alcohol are the major risk factors for oral squamous cell carcinoma (OSCC) [IARC, Monograph 85, 2004]. Betel nut chewing is linked to the development of oral [Zhang and Reichart, 2007] and esophageal cancer [Wu et al., 2006]. The anatomical sites of these two cancers are very closely related and oral habits (Chewing, smoking, alcohol) have a significant effect on the transformation of their normal epithelium to a malignant one.

The most well known risk factor for developing OSCC is the deleterious effects of tobacco. Indeed, OSCC was one of the first carcinomas to be linked with p53 mutations caused by tobacco usage [Brennan et al., 1995]. Alcohol use is synergistic with tobacco in causing OSCC. There are other cultural habit-forming risk factors that have an association with OSCC. Betel nut, a fruit that is the basic ingredient of a stimulant chew, is used in combination with betel leaf and slaked lime, with or without tobacco by an estimated 200 to 400 million people throughout Southeast Asia [Zain et al., 1997]. The odds ratio of developing leukoplakia and submucous fibrosis from using betel nut is five compared to one in non-chewers and the addition of tobacco raises the risk 3-fold [Warnakulasuriya et al., 2002]. The duration and frequency of betel nut use increase the risk of developing OSCC, suggesting a dose-
response relation [Lu et al., 1996]. Slaked lime included in betel quid causes inflammation in the sub-mucosal area. Calcium hydroxide content of lime in the presence of the areca nut is primarily responsible for the formation of reactive oxygen species that might cause oxidative damage in the DNA of buccal mucosa cells in betel quid chewers [Nair et al., 1990]. The major alkaloids of areca nut are arecoline, arecaidine, arecolidine, guvacoline and guvacine. Arecoline is the most abundant alkaloid of areca. These alkaloids undergo nitrosation and give rise to N-nitrosamines [Hoffmann et al., 1994]. It has been suggested that metabolic activation may involve the cytochrome p450 system [Sundqvist et al., 1991; Wary and Sharan, 1991]. The nitrosation of arecoline may produce a variety of betel quid-specific nitrosamines which may interact with DNA, proteins or other targets forming adduct to exert its carcinogenic activity.
Figure 1.2:  
A- Betel-nut trees  
B- Mature betel-nut  
C- Betel-nut in the market  
D- fresh, soft Betel Nut
**Figure 1.3:**

A - Alkaloids found in areca nut (Betel nut): (I) Arecoline, (II) Arecaidine, (XII) Guvacoline, (XIII) Guvacine;  
B - Areca Nut (Betel Nut) Derived N-Nitrosamines
Figure 1.4: Patient’s image of precancerous and cancerous lesion (Oral cavity) A- Lesion of hyperplastic cytology B- Lesion of dysplastic cytology and, C- Tumor outgrowth of squamous cell carcinoma cytology

Biology of Oral and Esophageal Cancer

Epidemiological evidence from case-control studies of Head and neck squamous cell carcinoma (HNSCC) indicates that a family history of Head and Neck cancer is a risk factor. Lip cancer is among the sites that show the strongest cancer clustering within families [Cannon-Albright et al., 1994]. The ability to repair DNA damaged by tobacco carcinogens, such as benzo-(α)-pyrene diol epoxide, is defective in some patients with Head and Neck cancer. These patients show an increased susceptibility to chromosome damage by mutagens [Spitz et al., 1989]. There are likely to be important familial clusters of individuals with more or less adequate genetic polymorphisms for carcinogen metabolizing and detoxifying enzymes [Jefferies et al., 1999]. In the early 1990’s, clinical observations and genetic studies of a variety of cancers led to the hypothesis that six genetic mutations were required to convert a normal somatic cell into a cancer cell [Hanahan and Weinberg, 2000]. These six mutations includes; self sufficiency for growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless ability to replicate, sustained angiogenesis, tissue invasion and metastasis.
Cancers occur as a consequence of alterations to genes that control growth and differentiation. DNA mutation leads to aberrant RNA and protein, with widespread deregulation of transcription during oncogenesis. The phenotypic outcomes of genetic changes in cancer have led to a general classification of cancer genes as either tumor suppressors, which are involved in inhibition of cell growth and survival, or oncogenes which promote these effects [Hanahan and Weinberg, 2000]. In most instances, multiple genes were altered, and the genes involved were different between neoplasms, which helped to explain the pathological heterogeneity observed in neoplasia. Various combinations of altered genes occurred, and if these accumulated at different rates, it might explain the unpredictability of premalignant lesions transforming into malignant ones [Fearon and Vogelstein, 1990]. It is well-established that an important part of the cancer etiology lies in stepwise accumulation of genetic changes [Hahn and Weinberg, 2002].

Oncogenes typically promote cellular proliferation. In almost all instances, there are copies of these genes in the normal human genome, which are involved in regulating ordinary growth. The key to carcinogenesis is that the proto-oncogene becomes oncogenes when mutated, amplified, or deregulated in such a way that it is overactive, and no longer controlled by usual feedback mechanisms. For example, the K-Ras gene can be activated by a point mutation that permits it to continue to stimulate cell proliferation even when the counter-regulatory signals are trying to halt the process. The c-Myc oncogene can be active in a tumor by gene amplification, so, even though each copy is potentially regulated, the excessive copy number fosters neoplastic growth. Oncogenes are considered to act “dominantly”, since a single mutated copy
will dominate over the normal ("wild type") copy of the gene, and only one mutation is sufficient to drive cell growth [Lengauer et al., 1998].

Tumor suppressor genes (TSGs) are present in all cells, and serve to regulate normal proliferation, or mediate cell-specific differentiation. These genes become involved in carcinogenesis through their inactivation. We inherit two copies of every gene – one from each parent – and both copies of a TSG must be inactivated to participate in carcinogenesis. There are multiple ways to inactivate TSGs, including point mutations, deletions (which may be called "loss of heterozygosity", or LOH), and silencing of the promoter by methylation of the cytosine residues at C-G sequences, which are preferentially present in promoters (in what are called "CpG islands"). When a sufficient number of the C-G’s in a promoter become methylated, the gene is silenced. Importantly, the methylation is passed on to the progeny of that cell, keeping that gene “permanently” silenced [Issa, 2004]. The TSG story can become a bit more complex. In some instances, a mutation in one copy of a TSG creates an altered protein that functionally inactivates the other copy (from the wild type allele), causing a “dominant negative” effect, which removes the tumor suppressor activity from that cell by a single mutation [Boland, 2008].

The classic mechanism of tumor suppressor gene inactivation is described by the two-hit model in which one allele is mutated and the other allele is lost through a number of possible mechanisms, resulting in loss of heterozygosity (LOH) at multiple loci [Knudson, 1985; Brown, 1997].
Figure 1.5: Diagrammatic illustration of mechanism of Microsatellites alterations
LOH can arise by a variety of genetic mechanisms, including physical deletion, chromosome nondisjunction, mitotic nondisjunction followed by reduplication of the remaining chromosome, mitotic recombination and gene conversion. LOH is one example of allelic imbalance that can arise from the complete loss of an allele or from an increase in copy number of one allele relative to the other. Allelic imbalances can be detected by measuring the proportion of one allele relative to the other in cells from individuals that are constitutionally heterozygous at a given locus. LOH involves complete loss of one of the two alleles at a locus, but normal cell contamination can confound the distinction between true LOH and other mechanisms of allelic imbalance. However, studies using Laser capture micro dissection, flow-cytometrically purified samples have shown that complete LOH can be clearly detected in tissue samples.

Microsatellites are tandem (di-, tri-, tetra-) nucleotide repeats generally located within noncoding areas of the genome [Shamoo, 2003]. They can have variable length and have been mapped to specific chromosomal regions, allowing for detection of adjacent genes of interest. Using simple PCR based techniques, one can identify if there is loss of genetic material, represented by complete deletion, or loss, of one allele (also known as loss of heterozygosity or LOH) [Boige et al., 1997; Paulson et al., 1999]. Genetic change not only plays a significant role in tumorigenesis, but it is also associated with inter- and intratumor heterogeneity [Bayani et al., 2007]. A major challenge facing cancer researchers today is to understand how genomic change can lead to the acquisition of such cellular heterogeneity. Genome-wide detection of chromosomal changes, including loss of heterozygosity (LOH) and copy number alterations (CNA), either gain or loss, are the focus of substantial attention in cancer
research. LOH is frequently observed in a variety of human cancers, and regions with frequent LOH may contain tumor suppressor genes. In addition, LOH may associate with the regions affected by haplo-insufficiency of a group of genes. Thus, detection of LOH will likely remain a cornerstone for predicting tumor aggressiveness for many human tumors [Maris et al., 2005]. New models of oncogenic progression must consider the combined effect of epigenetic and genetic change and concomitant causation of tumor heterogeneity [Balmain et al., 2003]. The term epigenetics was first introduced by a British embryologist and geneticist Conrad Hal Waddington in 1940, and was used to describe the study of the causal analysis of development [Slack, 2002]. Today, epigenetics refers to the study of heritable changes in gene expression without the change in gene sequence. These heritable changes are propagated as covalent chemical changes to the cytosine bases and are referred to as DNA methylation. DNA methylation, occurring predominantly in CpG dinucleotides, is an important epigenetic modification of the genome that is involved in mediating various cellular processes [Robertson, 2005]. DNA methylation has a wide range of biological functions, including an essential developmental role in the reprogramming of germ cells and early embryos, the genomic imprinting, the X chromosome inactivation, the repression of endogenous retrotransposons and the generalized role in gene expression [Scarano et al., 2005]. Abnormal methylation of DNA may result in increased transcription of oncogenes or silencing of tumor suppressor genes and is common in a variety of human cancer cells [Esteller, 2005]. Although the ramifications of global hypomethylation for tumor development are less well understood, it might contribute to chromosomal instability and then increases in gene expression [Feinberg et al., 2004; Baylin et al., 2006].
In 2004, the International Agency for Research on Cancer confirmed areca nut and betel quid as Class I human carcinogens with sufficient evidence of increased risk of precancerous oral fibrosis and cancer of the oral cavity, pharynx, and esophagus. Betel quid without tobacco, composed of betel nut, betel leaf and slaked lime, chewing has strong contribution to formation of oral submucous fibrosis (OSF), particularly in Taiwan. Oral submucous fibrosis (OSF) develops in lamina propria and connective tissues of oral cavity. Betel quid (BQ) chewing is a prevalent habit in India and many Southeast Asian countries [IARC, Monograph 85, 2004]. Cancer data from both population- and hospital based cancer registries in India have reported the highest incidence of esophageal cancer in Assam (age-adjusted rate of 33/100 000 males) in the north–east of the country, followed by Bangalore and Bombay. Betel nut chewing with or without tobacco has been shown to be independently associated with the development of esophageal cancer in Assam [Phukan et al., 2001].

Our particular concern is related with the East Khasi Hills district tribes in Meghalaya state of this region with one of the highest incidence rates of oral, oropharyngeal and esophageal cancer in the country (Stich et al., 1983). The variety of betel nut chewed by Khasi tribes and other communities here, locally known as ‘Kwai’, is raw, wet, unprocessed and consumed with betel-leaf and slaked lime without tobacco. The constituents of ‘Kwai’, show higher alkaloids, polyphenols and tannins as compared to the dried varieties of betel nut [Sharan 1996]. Arecoline is the main alkaloid of raw betel nut (RBN) or areca nut. The genotoxic potentiality of arecoline in mammalian cells [Deb and Chatterjee 1998, Chatterjee and Deb 1999] and the genotoxicity of saliva of Kwai-chewers among the tribal population of Meghalaya state were demonstrated in Chinese hamster ovary cells [Stich et al., 1985]. It has been earlier
reported by our research group that heavy chewers of betel-nut (BN) in the East Khasi Hills district show higher DNA damage, delay in cell kinetics, p53 expression and lower GSH-level than non-chewers [Kumpawat and Chatterjee, 2003]. The characterization of these cytotoxicity and genotoxicity of arecoline indeed has highlighted the potential risk of arecoline exposure in betel chewers, nevertheless, molecular-based interpretation of betel nut use in the initiation and progression events of OSCC & ESCC are still not well understood. One of the major challenges in cancer diagnosis is the use of cancer-specific markers for the early detection of sporadic cancer. Although the analysis of genetic changes that target specific gene alterations provide an accurate molecular basis for assessment of the cancer stage, most of these alterations could only be detected at an advanced stage and could be a laborious and expensive undertaking with a low return in treating the disease.

It is evident from various studies that a consistent LOH could be used as an indicator for the targeted deletion of tumor suppressor genes. Thus, LOH of critical chromosomal regions could manifest susceptibility to or the presence of cancer and play an etiologic role in its initiation and progression to carcinoma. Furthermore, LOH could also be observed in neoplastic and apparent phenotypically normal preneoplastic cells that may eventually progress to become cancer [Pan et al., 2005]. The hypermethylation of CpG islands in gene promoter regions is associated with aberrant silencing of transcription and has been regarded as a common mechanism for inactivation of tumor suppressor genes in human cancer [Herman et al., 2003]. As compared with normal cells, the malignant cells show major disruptions in their DNA methylation patterns [Baylin et al., 2000].
Role of p16, pRb and p53 Tumor Suppressor Genes in Human Cancers

A few genomic regions of loss have been implicated as early events in the formation of preneoplastic lesions. These include regions on chromosomal arms 9p, 17p and 3p. The genes involved in early genesis of OSCC, which map to these regions of frequent loss, include \textit{p14ARF} and \textit{p16INK4a} on 9p21, TP53 on 17p13, and RB on chromosome 13q14.

\textbf{p16INK4a / p14ARF}— In mammalian systems, the maintenance of homeostasis requires a tight control of cell proliferation. p16INK4a (hereafter p16) inhibits the cyclin-dependent kinases CDK4 and CDK6, thereby keeping the retinoblastoma protein (pRB) in a hypophosphorylated state and arresting cells in the G1 phase of the division cycle. Loss or inactivation of the INK4a/ARF locus (harboring p16 and ARF) are among the most frequent alterations seen in human cancers, underscoring the widely recognized role of p16 as a tumor suppressor. The p16 gene is involved in the regulation of the RB-pathway. Product of the alternate transcript, \textit{p14ARF}, interacts with MDM2, which is involved in degradation of TP53. Loss of the INK4a/ARF locus results in deregulation of both, p53- and pRB- pathways and hence uncontrolled cell proliferation [Lal et al., 2008]

\textbf{p53}—Normal p53 function is lost in almost all tumors, either through mutations or genomic deletions of the gene or through up-regulation of negative regulators of p53 function. LOH for the p53 locus has been reported in about 60% of OSCC. It is almost impossible to detect p53 expression in normal tissue due to a high turnover of the p53 protein. This expression pattern of p53 is altered in tumor tissue wherein mutant forms of p53, which are highly stable, are readily detected by immunohistochemical analyses. Approximately 50% OSCC tumors stain positive for
p53. Another member of the p53- family implicated in OSCC genesis and progression is p63. p63 is thought to play a role in differentiation of keratinocytes and epithelial cells. Decreased p63 expression in OSCC, in the context of p53 expression, has been shown to correlate with increased metastatic load and decreased overall survival [de Oliveira et al., 2007].

**pRB** - The Rb gene, located on chromosome 13q14.2, was the first tumor suppressor gene to be identified in humans and was initially determined to be associated with the development of retinoblastoma [Friend et al., 1986]. Rb encodes a cell cycle control protein that is at the convergence of several positive and negative regulatory pathways that are often referred to collectively as the Rb pathway [Sherr, 1994]. Hypophosphorylated pRb, which is regarded as the active form, can form stable complex with various transcriptional activators of the E2F family and halt the cell cycle progression during G1 [Chellappan et al., 1991]. Suppression of pRb function through hyperphosphorylation causes the release of the E2F factors and triggers a burst of gene expressions that facilitates G1-S transition [Buchkovich et al., 1989; Johnson, et al., 1993]. Functional loss of the Rb gene frequently occurs in the carcinogenic processes of many types of cancer [Benedict et al., 1990]. LOH at the Rb locus is an important event reflecting potential functional alteration in the Rb gene. LOH on 13q, where Rb is located, is a common feature in many types of cancer involving bladder, lung, breast, head and neck, and other organs [Xu et al., 1993; Tamura et al., 1997; Borg et al., 1992; Yoo et al., 1994; Pei et al., 1995; Peng et al., 1995; Zhang et al., 1994]. In human esophageal cancer, LOH at the Rb locus was observed in 54% of SCCs and in 36% of adenocarcinomas [Boynton et al., 1991; Huang et al., 1992].
**Figure 1.6: A- p53 tumor suppressor pathway.** p53 protein is latent in cells and can be activated by cellular stress signals such as hypoxia, DNA damage, and inappropriate oncogene signaling. In response to these stresses, p53 accumulates and transcriptionally up-regulates genes involved in G1 and G2-M arrest, such as the cdk inhibitor p21/waf1 and 14–3-3σ, respectively. Alternatively, p53 can transactivate the proapoptotic genes bax, NOXA, p53AIP, and PUMA, which are involved in mitochondrial apoptotic signaling, or fas and KILLER/DR5, which play a role in death receptor signaling.

**B- p16INK4a growth suppressive pathway.** p16INK4a protein interacts with cdk4 and/or cdk6 to inhibit cyclin D-binding and kinase activity. This allows the cdk4/6 substrate pRB to accumulate in hypophosphorylated form, which binds and inhibits the activity of E2F/DP transcription factors, whose transcriptional targets are necessary for the G1 to S (synthesis) phase transition of the cell cycle.

LOH at chromosome 17p13, the location of the locus for p53, has been suggested as a later event than LOH at 9p21 for HNSCC [Califano et al., 1996]. Studies of genetic progression have suggested that p53 alteration occurs at greater frequency in invasive carcinomas than in noninvasive lesions [Boyle et al., 1993]. Several studies have demonstrated that 40–70% of SCCHN contain mutations in exons 5–9 at the p53 locus [Shin et al., 1996; Olshan et al., 1997]. Wild-type p53 protein has numerous
functions including gene transcription, DNA synthesis and repair, and apoptosis [Sionov and Haupt, 1999].

One of the earliest known events in head and neck squamous cell carcinogenesis may occur at chromosome 9p21 [Califano et al., 1996; El-Naggar, 1999]. This chromosome contains the locus for two proteins, ARF and p16 [Zhang et al., 1998]. The p16 protein exerts a tumor suppressor function by binding to the cyclin D1 CDK4/CDK6 complex preventing phosphorylation of the retinoblastoma protein, resulting in G1 arrest [Lai and El-Naggar, 1999]. Inactivation of p16 may occur through several different mechanisms including homozygous deletion, methylation of the gene promoter with subsequent transcriptional silencing, and single base pair mutation [Gonzalez et al., 1997; El-Naggar et al., 1997]. The most frequent method of inactivation is homozygous deletion [Reed et al., 1996].

An association between aberrant pRb and p53 expression was observed in bladder and several other types of cancer [Cordon-Cardo et al., 1997; Cote et al., 1998]. p53 is critical for coordinating multiple growth control checkpoints in response to genotoxic insults and abnormal proliferation [Kastan et al., 1991]. Wild-type p53 can block malignant cell transformation by inhibiting proliferation, facilitating DNA repair, and stimulating apoptosis in genetically injured cells [Gotz and Montenarh, 1996]. Intuitively, an increased proliferation capacity due to Rb loss together with a decreased rate of apoptosis due to p53 alteration would greatly enhance the tumorigenic potential of the affected cells. Indeed, recent studies in the murine system showed that, although germ-line mutation in Rb or p53 resulted in predisposition to cancer [Donehower et al., 1992], heterozygous mice mutant for both Rb and p53 showed reduced viability and novel cancer pathology, including increased cancer
burden [Williams et al., 1994]. Loss of Rb can activate apoptosis at least in part via elevated p53 expression, and loss of p53 gene function prevents cell death in the central nervous system of Rb-mutated mouse embryos [Macleod et al., 1996]. Therefore, it is possible that inactivation of both Rb and p53 genes in the cell produces a synergistic effect, which imposes stronger selective pressure for cellular transformation. Indeed, it was reported that, in cells that sustained lesion in the Rb pathway, there was a strong selection for the loss or inactivation of wild-type p53 [Sherr, 1996].

From background description now we have a broad overview about the various factors involved in the initiation and progression of ESCC and OSCC. Oral as well as esophageal squamous cell carcinoma (OSCC and ESCC) is the most common cancers in India and their highest incidence has been reported in north-eastern states of India [Stoner and Gupta, 2001]. The morbidity and mortality associated with this disease is a major concern in this region. Of several factors that are implicated for these cancers, consumption of raw betel-nut (Areca catechu L.) appears to be highly relevant besides the use of tobacco and alcohol. In the State of Meghalaya, the variety of Areca nut chewed, locally called ‘kwai’, is raw, unprocessed with higher contents of alkaloids, polyphenol and tannins as compared to the dried one [Sharan, 1996]. The betel-quad used in Meghalaya contains raw betel-nut, lime paste and small portion of betel-leaf without tobacco and other constituents. It was reported that betel quid chewing with or without tobacco is associated with the development of esophageal cancer in Assam [Phukan et al., 2001]. The genotoxic potential of arecoline (alkaloid of RBN) in mammalian cells [Deb and Chatterjee, 1998; Chatterjee and Deb, 1999] and the genotoxicity of RBN in RBN heavy-chewers than non-chewers in Meghalaya were
demonstrated earlier [Kumpawat and Chatterjee, 2003], however no attempt has been made to understand the genomic changes induced by RBN leading to cancer. In the Indian population, a great majority of OSCCs and ESCCs are associated with chronic tobacco consumption [Braakhuis et al., 2003]. Due to this fact, it has been difficult to assess the effects of purely and predominantly RBN induced genetic alterations without interference of other confounding factors like tobacco smoking or chewing. Therefore, present study aimed for determining whether RBN can alone induces these cancers and if yes, then, are the above mentioned genes involved in the development of these cancers? The present investigation was carried out in 99 OSCC and ESCC samples of which 30 samples collected from patients with only RBN-chewing habit. Our results demonstrate the significant involvement of 9p21 region where CDKN2A gene is present and also 9p23 region in RBN-induced carcinogenesis. Reports on the deregulation of molecular networks associated with raw betel nut (RBN)-induced cancers are scanty. The present study is designed to understand the role of RBN in ESCC and OSCC development in cancer patients with RBN chewing habit. There are two main objectives, first one is to analyze the genetic alterations (LOH) and the second one to investigate epigenetic (promoter hypermethylation) events.
The objectives of this study are as follows:

- Identification of critical regions of Loss of Heterozygosity (LOH) at chromosome arms 9p, 13q and 17p by deletion mapping using PCR based techniques for highly polymorphic microsatellite markers to understand the deregulation of p16INK4A, pRb and p53 genes in tumor samples collected from RBN chewers.

- Detection of promoter hypermethylation in p16INK4A and RB1 genes by using methylation- specific PCR (MS – PCR) in tumor samples collected from RBN chewers.